

=> s G-CSF (p) (dmier? or trimer or tetramer? or multimer?)
L4 341 G-CSF (P) (DMIER? OR TRIMER OR TETRAMER? OR MULTIMER?)

=> s l4 (p) (conjugat? or PEG)
L5 13 L4 (P) (CONJUGAT? OR PEG)

=> d l5 1-13 bib

L5 ANSWER 1 OF 13 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2002:353487 CAPLUS
DN 136:364900
TI Construction, cloning, recombinant expression and therapeutic use of
single-chain dimeric granulocyte colony-stimulating factor and other
single-chain multimeric protein conjugates
IN Nissen, Torben Lauesgaard; Jensen, Anne Dam
PA Maxygen Aps, Den.; Maxygen Holdings Ltd.
SO PCT Int. Appl., 108 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002036626	A1	20020510	WO 2001-DK724	20011101
	W:				
				AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,	
				CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,	
				GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,	
				LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL,	
				PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG,	
				UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM	
	RW:				
				GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,	
				DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF,	
				BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG	
	AU 2002012108	A5	20020515	AU 2002-12108	20011101
	US 2002142964	A1	20021003	US 2001-3496	20011101
	EP 1334127	A1	20030813	EP 2001-980207	20011101
	R:				
				AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,	
				IE, SI, LT, LV, FI, RO, MK, CY, AL, TR	
PRAI	DK 2000-1647	A	20001102		
	US 2000-245727P	P	20001102		
	WO 2001-DK724	W	20011101		

RE.CNT 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 2 OF 13 USPATFULL on STN
AN 2004:24747 USPATFULL
TI Method for refolding proteins containing free cysteine residues
IN Rosendahl, Mary S., Broomfield, CO, UNITED STATES
Cox, George N, Louisville, CO, UNITED STATES
Doherty, Daniel H, Boulder, CO, UNITED STATES
PI US 2004018586 A1 20040129
AI US 2003-276358 A1 20030410 (10)
WO 2001-US16088 20010516
DT Utility
FS APPLICATION
LREP SHERIDAN ROSS PC, 1560 BROADWAY, SUITE 1200, DENVER, CO, 80202
CLMN Number of Claims: 55
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 5001

L5 ANSWER 3 OF 13 USPATFULL on STN
AN 2003:237907 USPATFULL
TI Compositions and methods for the therapy and diagnosis of colon cancer

IN King, Gordon E., Shoreline, WA, UNITED STATES
Meagher, Madeleine Joy, Seattle, WA, UNITED STATES
Xu, Jiangchun, Bellevue, WA, UNITED STATES
Secrist, Heather, Seattle, WA, UNITED STATES
Jiang, Yuqiu, Kent, WA, UNITED STATES
PA Corixa Corporation, Seattle, WA, UNITED STATES, 98104 (U.S. corporation)
PI US 2003166064 A1 20030904
AI US 2002-99926 A1 20020314 (10)
RLI Continuation-in-part of Ser. No. US 2001-33528, filed on 26 Dec 2001,
PENDING Continuation-in-part of Ser. No. US 2001-920300, filed on 31 Jul
2001, PENDING
PRAI US 2001-302051P 20010629 (60)
US 2001-279763P 20010328 (60)
US 2000-223283P 20000803 (60)
DT Utility
FS APPLICATION
LREP SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVE, SUITE 6300,
SEATTLE, WA, 98104-7092
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 8531
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 4 OF 13 USPATFULL on STN
AN 2003:106233 USPATFULL
TI Compositions and methods for the therapy and diagnosis of pancreatic
cancer
IN Benson, Darin R., Seattle, WA, UNITED STATES
Kalos, Michael D., Seattle, WA, UNITED STATES
Lodes, Michael J., Seattle, WA, UNITED STATES
Persing, David H., Redmond, WA, UNITED STATES
Hepler, William T., Seattle, WA, UNITED STATES
Jiang, Yuqiu, Kent, WA, UNITED STATES
PA Corixa Corporation, Seattle, WA, UNITED STATES, 98104 (U.S. corporation)
PI US 2003073144 A1 20030417
AI US 2002-60036 A1 20020130 (10)
PRAI US 2001-333626P 20011127 (60)
US 2001-305484P 20010712 (60)
US 2001-265305P 20010130 (60)
US 2001-267568P 20010209 (60)
US 2001-313999P 20010820 (60)
US 2001-291631P 20010516 (60)
US 2001-287112P 20010428 (60)
US 2001-278651P 20010321 (60)
US 2001-265682P 20010131 (60)
DT Utility
FS APPLICATION
LREP SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVE, SUITE 6300,
SEATTLE, WA, 98104-7092
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 14253
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 5 OF 13 USPATFULL on STN
AN 2003:102452 USPATFULL
TI Site protected protein modification
IN Pettit, Dean K., Seattle, WA, United States
PA Immunex Corporation, Seattle, WA, United States (U.S. corporation)
PI US 6548644 B1 20030415
AI US 1997-814305 19970310 (8)
DT Utility

FS GRANTED
EXNAM Primary Examiner: Low, Christopher S. F.; Assistant Examiner: Mohamed, Abdel A.
LREP Henry, Janis C.
CLMN Number of Claims: 13
ECL Exemplary Claim: 1
DRWN 3 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 1168
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 6 OF 13 USPATFULL on STN
AN 2003:31098 USPATFULL
TI Site specific protein modification
IN Pettit, Dean K., Seattle, WA, UNITED STATES
PA Immunex Corporation (U.S. corporation)
PI US 2003023049 A1 20030130
AI US 2002-243230 A1 20020912 (10)
RLI Continuation of Ser. No. US 1998-102530, filed on 22 Jun 1998, GRANTED, Pat. No. US 6451986
DT Utility
FS APPLICATION
LREP IMMUNEX CORPORATION, LAW DEPARTMENT, 51 UNIVERSITY STREET, SEATTLE, WA, 98101
CLMN Number of Claims: 18
ECL Exemplary Claim: 1
DRWN 1 Drawing Page(s)
LN.CNT 1246
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 7 OF 13 USPATFULL on STN
AN 2002:259392 USPATFULL
TI Single-chain polypeptides
IN Nissen, Torben Lauesgaard, Frederiksberg C, DENMARK
Jensen, Anne Dam, Copenhagen, DENMARK
PI US 2002142964 A1 20021003
AI US 2001-3496 A1 20011101 (10)
PRAI US 2000-245727P 20001102 (60)
DT Utility
FS APPLICATION
LREP Joanne Petithory, Maxygen, Inc., 515 Galveston Drive, Redwood City, CA, 94063
CLMN Number of Claims: 33
ECL Exemplary Claim: 1
DRWN 2 Drawing Page(s)
LN.CNT 3866
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 8 OF 13 USPATFULL on STN
AN 2002:243051 USPATFULL
TI Compositions and methods for the therapy and diagnosis of ovarian cancer
IN Algate, Paul A., Issaquah, WA, UNITED STATES
Jones, Robert, Seattle, WA, UNITED STATES
Harlocker, Susan L., Seattle, WA, UNITED STATES
PA Corixa Corporation, Seattle, WA, UNITED STATES, 98104 (U.S. corporation)
PI US 2002132237 A1 20020919
AI US 2001-867701 A1 20010529 (9)
PRAI US 2000-207484P 20000526 (60)
DT Utility
FS APPLICATION
LREP SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVE, SUITE 6300, SEATTLE, WA, 98104-7092
CLMN Number of Claims: 11
ECL Exemplary Claim: 1
DRWN No Drawings

LN.CNT 25718

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 9 OF 13 USPATFULL on STN
AN 2002:242791 USPATFULL
TI Compositions and methods for the therapy and diagnosis of colon cancer
IN King, Gordon E., Shoreline, WA, UNITED STATES
Meagher, Madeleine Joy, Seattle, WA, UNITED STATES
Xu, Jiangchun, Bellevue, WA, UNITED STATES
Secrist, Heather, Seattle, WA, UNITED STATES
PA Corixa Corporation, Seattle, WA, UNITED STATES (U.S. corporation)
PI US 2002131971 A1 20020919
AI US 2001-33528 A1 20011226 (10)
RLI Continuation-in-part of Ser. No. US 2001-920300, filed on 31 Jul 2001,
PENDING
PRAI US 2001-302051P 20010629 (60)
US 2001-279763P 20010328 (60)
US 2000-223283P 20000803 (60)
DT Utility
FS APPLICATION
LREP SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVE, SUITE 6300,
SEATTLE, WA, 98104-7092
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 8083
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 10 OF 13 USPATFULL on STN
AN 2002:217388 USPATFULL
TI Site specific protein modification
IN Pettit, Dean K., Seattle, WA, United States
PA Immunex Corporation, Seattle, WA, United States (U.S. corporation)
PI US 6441136 B1 20020827
AI US 2000-580181 20000526 (9)
RLI Continuation of Ser. No. US 1998-102530, filed on 22 Jun 1998
DT Utility
FS GRANTED
EXNAM Primary Examiner: Eyler, Yvonne; Assistant Examiner: Brannock, Michael
LREP Henry, Janis C.
CLMN Number of Claims: 8
ECL Exemplary Claim: 1
DRWN 1 Drawing Figure(s); 1 Drawing Page(s)
LN.CNT 1193
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 11 OF 13 USPATFULL on STN
AN 2002:202251 USPATFULL
TI Site specific protein modification
IN Pettit, Dean K., Seattle, WA, United States
PA Immunex Corporation, Seattle, WA, United States (U.S. corporation)
PI US 6433158 B1 20020813
AI US 2000-580235 20000526 (9)
RLI Continuation of Ser. No. US 1998-102530, filed on 22 Jun 1998
DT Utility
FS GRANTED
EXNAM Primary Examiner: Eyler, Yvonne; Assistant Examiner: Brannock, Michael
LREP Henry, Janis C.
CLMN Number of Claims: 5
ECL Exemplary Claim: 1
DRWN 1 Drawing Figure(s); 1 Drawing Page(s)
LN.CNT 1232
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 12 OF 13 USPATFULL on STN
 AN 2002:156714 USPATFULL
 TI SITE SPECIFIC PROTEIN MODIFICATION
 IN PETTIT, DEAN K., SEATTLE, WA, UNITED STATES
 PI US 2002081309 A1 20020627
 US 6451986 B2 20020917
 AI US 1998-102530 A1 19980622 (9)
 DT Utility
 FS APPLICATION
 LREP IMMUNEX CORPORATION, LAW DEPARTMENT, 51 UNIVERSITY STREET, SEATTLE, WA,
 98101
 CLMN Number of Claims: 18
 ECL Exemplary Claim: 1
 DRWN 1 Drawing Page(s)
 LN.CNT 1247
 CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 13 OF 13 USPATFULL on STN
 AN 92:92928 USPATFULL
 TI Cysteine added variants of interleukin-3 and chemical modifications
 thereof
 IN Shaw, Gray, Bedford, MA, United States
 Veldman, Geertruida, Sudbury, MA, United States
 Wooters, Joseph L., Brighton, MA, United States
 PA Genetics Institute, Cambridge, MA, United States (U.S. corporation)
 PI US 5166322 19921124
 AI US 1989-341990 19890421 (7)
 DT Utility
 FS Granted
 EXNAM Primary Examiner: Draper, Garnette D.
 LREP Cserr, Luann, Eisen, Bruce
 CLMN Number of Claims: 9
 ECL Exemplary Claim: 1
 DRWN 3 Drawing Figure(s); 3 Drawing Page(s)
 LN.CNT 886
 CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d 14 1-13 kwic

L4 ANSWER 1 OF 341 MEDLINE on STN
 AB . . . of CD38-expressing cells, with or without depletion of B-cell
 Ag-expressing cells. Using myeloma BM or blood cells diluted into
 allogeneic **G-CSF** primed leukapheresis cells,
 bispecific **tetrameric** Ab complexes that bind dextran iron
 particles were used to label and retain cells in a magnetic column,
 StemSep. Depletion. . .

L4 ANSWER 2 OF 341 MEDLINE on STN
 AB . . . blast cells taken at presentation from nine children with ALL.
 Blast cells were expanded in serum-free medium supplemented with Flt3L,
G-CSF, GM-CSF, IL-3, IL-6 and SCF for 7 days and
 subsequently stimulated with Flt3L, GM-CSF and TGF-beta for a further 14.
 . . HLA-A*02-positive ALL pulsed with CMV-associated peptides could
 induce significant proliferation of peptide-specific CD8+ T cells. This
 specificity was verified using **tetrameric** complexes of HLA class
 I/antigenic peptide. DC could also be generated from cells taken at times
 of complete remission of. . .

L4 ANSWER 3 OF 341 MEDLINE on STN
 AB The granulocyte colony-stimulating factor receptor (**G-**
CSF-R) forms a **tetrameric** complex with **G-**
CSF containing two ligand and two receptor molecules. The
 N-terminal Ig-like domain of the **G-CSF-R** is required

for receptor dimerization, but it is not known whether it binds **G-CSF** or interacts elsewhere in the complex. Alanine scanning mutagenesis was used to show that residues in the Ig-like domain of the **G-CSF-R** (Phe(75), Gln(87), and Gln(91)) interact with **G-CSF**. This binding site for **G-CSF** overlapped with the binding site of a neutralizing anti-**G-CSF-R** antibody. A model of the Ig-like domain showed that the binding site is very similar to the viral interleukin-6 binding site (site III) on the Ig-like domain of gp130, a related receptor. To further characterize the **G-CSF-R** complex, exposed and inaccessible regions of monomeric and dimeric ligand-receptor complexes were mapped with monoclonal antibodies. The results showed that the E helix of **G-CSF** was inaccessible in the dimeric but exposed in the monomeric complex, suggesting that this region binds to the Ig-like domain of the **G-CSF-R**. In addition, the N terminus of **G-CSF** was exposed to antibody binding in both complexes. These data establish that the dimerization interface of the complete receptor complex is different from that in the x-ray structure of a partial complex. A model of the **tetrameric G-CSF.G-CSF-R** complex was prepared, based on the viral interleukin-6.gp130 complex, which explains these and previously published data.

L4 ANSWER 4 OF 341 MEDLINE on STN
 AB . . . retroviral construct were stably expressed, processed, and presented in the context of HLA class I molecules. CD34(+) cells isolated from **G-CSF** mobilized peripheral blood were transduced with high efficiency (40-60%) with this retroviral construct. These cells could be considerably expanded in. . . the context of HLA-A2, demonstrating the antigen-specific CTL priming capacity of retrovirally transduced DC. Staining of the T cells with **tetramers** of HLA-A2 and the influenza virus peptide demonstrated a marked antigen-specific CTL enrichment after 2 in vitro stimulations using DC. . . However, additional in vitro stimulations of the T cells with transduced DC did not result in a further enrichment of **tetramer** staining cells.
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L4 ANSWER 5 OF 341 MEDLINE on STN
 AB Granulocyte colony-stimulating factor (**G-CSF**) forms a **tetrameric** complex with its receptor, comprising two **G-CSF** and two receptor molecules. The structure of the complex is unknown, and it is unclear whether there are one or two binding sites on **G-CSF** and the receptor. The immunoglobulin-like domain and the cytokine receptor homologous module of the receptor are involved in **G-CSF** binding, and Arg288 in the cytokine receptor homologous module is particularly important. To identify residues in **G-CSF** that interact with Arg288, selected charged residues in **G-CSF** were mutated to Ala. To clarify whether there are two binding sites, a chimeric receptor was created in which the Ig domain was replaced with that of the related receptor gp130. This chimera bound **G-CSF** but could not transduce a signal, consistent with failure of dimerization and loss of one binding site. The **G-CSF** mutants had reduced mitogenic activity on cells expressing wild-type receptor. When tested with the chimeric receptor, all **G-CSF** mutants except one (E46A) showed reduced binding, suggesting that Glu46 is important for interaction with the Ig domain. On cells expressing R288A receptor, all the **G-CSF** mutants except E19A showed reduced mitogenic activity, indicating that Glu19 of **G-CSF** interacts with Arg288 of the receptor.

L4 ANSWER 6 OF 341 MEDLINE on STN
 AB Expression and purification of the extracellular portion of granulocyte colony-stimulating factor (**G-CSF**) receptor, which

contains an immunoglobulin-like (Ig) domain and the cytokine receptor homologous (CRH) region, using a baculovirus secretion system have shown that a **tetrameric** Ig-CRH protein (about 200 kDa) existed in addition to the dimer (85 kDa) [7]. Scatchard analysis revealed that the **tetramer** had ligand binding affinity, with a dissociation constant of about 2.5 nM. The **tetramer** dissociated into monomers at pH 2 and was re-formed at pH7, in contrast, the dimer was re-dimerized with the same treatment. These observations led us to hypothesize the existence of conformational heterogeneity, which leads to **tetramer** as well as dimer formation, in the soluble state of the Ig-CRH protein.

L4 ANSWER 7 OF 341 MEDLINE on STN

AB An extracellular portion of granulocyte colony-stimulating factor (**G-CSF**) receptor, which contains an immunoglobulin-like (Ig) domain and cytokine receptor homologous (CRH) region, was secreted into the medium using Trichoplusia. . . ni-Autographa californica nuclear polyhedrosis virus system. The gene product was purified to homogeneity mainly as a dimer (85 kDa) using **G-CSF** affinity column chromatography and gel filtration HPLC, although the product existed as a monomer (45 kDa) in the medium. Scatchard. . . (Kd = about 100 pM), which is comparable with the Kd value of the cell surface receptor. The binding of **G-CSF** to Ig-CRH induced its **tetramerization** (200-250 kDa). The molecular composition of the **tetrameric** complex showed a stoichiometry of four ligands bound to four Ig-CRH. These results suggested that the oligomeric mechanism of the **G-CSF** receptor differs from that reported for growth hormone (GH) receptor, although CD spectrum spectroscopy suggested that the Ig-CRH has a. . .

L4 ANSWER 8 OF 341 MEDLINE on STN

AB The expression of the mouse gene (**G-CSF**) encoding granulocyte colony-stimulating factor is controlled by at least three regulatory elements, GPE1, GPE2 and GPE3 (**G-CSF** promoter elements). A set of 30-mer oligodeoxyribonucleotides (oligos) scanning the GPE3 region (-104 to -51) of the **G-CSF** promoter was synthesized, and the **tetramer** of each oligo was inserted upstream from the cat gene with the simian virus 40 enhancer element. By introducing these. . . in BAM3 cells by lipopolysaccharide. The results suggest that these nuclear factors play important roles in the constitutive expression of **G-CSF** in CHU-2 cells and its inducible expression in macrophages.

L4 ANSWER 9 OF 341 MEDLINE on STN

AB . . . and cytokines have come under scientific scrutiny. Recently receptors for IL-2 alpha, IL-2 beta, IL-3, IL-4, IL-5, IL-6, IL-7, erythropoietin, **G-CSF** and GM-CSF have been isolated and cloned. It has become apparent that they have structural homology that is shared by. . . low affinity binding forms exist for all these receptors. Binding affinity may depend on the formation of receptor heterodimers or **multimers**, association with other membrane proteins or differential glycosylation. Soluble receptor forms have been described for IL-2 alpha, IL-4, IL-5, IL-6. . .

L4 ANSWER 10 OF 341 MEDLINE on STN

AB At least three regulatory elements GPE1, GPE2 and GPE3 (**G-CSF** promoter elements) controlling the gene (**G-CSF**) encoding granulocyte colony-stimulating factor (**G-CSF**) are indispensable for the constitutive expression of the **G-CSF** gene in human CHU-2 cells and for its lipopolysaccharide(LPS)-inducible expression in macrophages. The enhancer activities of each regulatory element were examined with or without the SV40 enhancer element placed downstream from the reporter gene. A GPE1 **tetramer** mediated the constitutive expression in CHU-2 cells, and the LPS-inducible expression in macrophage cell lines, while the GPE2

element was active in CHU-2 and LPS-treated macrophage cell lines only in combination with the SV40 enhancer. A GPE3 **tetramer** had efficient enhancer activity in CHU-2 cells but not in macrophage cell lines without the SV40 enhancer. In combination with. . .

L4 ANSWER 11 OF 341 MEDLINE on STN

AB . . . products have been implicated. We have employed monoclonal antibody anti-T3B covalently coupled to CnBr-activated Sepharose 4B beads, to show that **multimeric** ligation of T cell antigen receptor leads to T cell receptiveness to interleukin 1 (IL-1), as indicated by T cell. . . of these findings, total RNA was extracted from T3B Sepharose-primed and IL-1-stimulated T lymphocytes and probed for granulocyte-monocyte-CSF (GM-CSF), granulocyte-CSF (**G-CSF**), and monocyte-CSF (M-CSF) mRNA. GM-CSF, but not **G-CSF** or M-CSF, messages were detected. Nuclear "run on" assays revealed that IL-1 action is effective primarily at the level of. . .

L4 ANSWER 12 OF 341 CAPLUS COPYRIGHT 2004 ACS on STN

AB The invention relates to single-chain **multimeric** polypeptides comprising at least two units of a monomeric polypeptide linked via a peptide bond or a peptide linker, wherein. . . one non-polypeptide moiety covalently bound to an attachment group of the polypeptide. The polypeptide is preferably a granulocyte colony-stimulating factor (**G-CSF**) dimer bound to a polymer mol., preferably to one or more polyethylene glycol (PEG) mols. Construction and cloning of a synthetic gene encoding single-chain **G-CSF** dimer, expression of the single-chain **G-CSF** dimer in *Saccharomyces cerevisiae* and in CHO cells, purification of the recombinant single-chain **G-CSF** dimers from yeast and CHO cells, and covalent attachment of SPA-PEG to the purified single-chain **G-CSF** dimers are described. In vitro biol. activity of non-conjugated and conjugated single-chain **G-CSF** dimers, and in vivo activity of the single-chain **G-CSF** dimers in healthy rats and in rats with chemotherapy-induced neutropenia are reported.

L4 ANSWER 13 OF 341 CAPLUS COPYRIGHT 2004 ACS on STN

AB The granulocyte colony-stimulating factor receptor (**G-CSF-R**) forms a **tetrameric** complex with **G-CSF** containing two ligand and two receptor mols. The N-terminal Ig-like domain of the **G-CSF-R** is required for receptor dimerization, but it is not known whether it binds **G-CSF** or interacts elsewhere in the complex. Alanine scanning mutagenesis was used to show that residues in the Ig-like domain of the **G-CSF-R** (Phe75, Gln87, and Gln91) interact with **G-CSF**. This binding site for **G-CSF** overlapped with the binding site of a neutralizing anti-**G-CSF-R** antibody. A model of the Ig-like domain showed that the binding site is very similar to the viral interleukin-6 binding site (site III) on the Ig-like domain of gp130, a related receptor. To further characterize the **G-CSF-R** complex, exposed and inaccessible regions of monomeric and dimeric ligand-receptor complexes were mapped with monoclonal antibodies. The results showed that the E helix of **G-CSF** was inaccessible in the dimeric but exposed in the monomeric complex, suggesting that this region binds to the Ig-like domain of the **G-CSF-R**. In addition, the N terminus of **G-CSF** was exposed to antibody binding in both complexes. These data establish that the dimerization interface of the complete receptor complex is different from that in the x-ray structure of a partial complex. A model of the **tetrameric G-CSF** · **G-CSF-R** complex was prepared, based on the viral interleukin-6·gp130 complex, which explains these and previously published data.